

WHAT IS CLAIMED IS:

1 1. A reaction mixture for producing a product saccharide, wherein the
2 reaction mixture comprises an acceptor saccharide and a first type of plant or microorganism
3 cell that produces: a) a nucleotide sugar, and b) a first recombinant glycosyltransferase that
4 catalyzes the transfer of a sugar from the nucleotide sugar to the acceptor saccharide to form
5 the product saccharide.

1 2. The reaction mixture of claim 1, wherein the cells are selected from one
2 or more of the group consisting of bacterial cells, yeast cells, fungal cells, and plant cells.

1 3. The reaction mixture of claim 1, wherein the cells are permeabilized or
2 otherwise disrupted.

1 4. The reaction mixture of claim 1, wherein the glycosyltransferase is a
2 fucosyltransferase and the nucleotide sugar is GDP-fucose.

1 5. The reaction mixture of claim 1, wherein the glycosyltransferase is a
2 sialyltransferase and the nucleotide sugar is CMP-sialic acid

1 6. The reaction mixture of claim 1, wherein nucleotide sugar is selected
2 from the group consisting of UDP-Gal, UDP-Glc, UDP-Glucuronic acid, UDP-GalNAc,
3 UDP-Galacturonic acid, GDP-mannose.

1 7. The reaction mixture of claim 1, wherein the first type of cell produces
2 the nucleotide sugar at an elevated level compared to a wild-type cell.

1 8. The reaction mixture of claim 7, wherein the elevated level of the
2 nucleotide sugar results from a deficiency in the ability of the cell to incorporate the
3 nucleotide sugar into a polysaccharide normally produced by the cell.

1 9. The reaction mixture of claim 7, wherein the elevated level of the
2 nucleotide sugar is at least 10% higher than the level of the nucleotide sugar produced by the
3 wild-type cell.

1 10. The reaction mixture of claim 9, wherein the elevated level of the
2 nucleotide sugar is at least 25% higher than the level of the nucleotide sugar produced by the
3 wild-type cell.

1 11. The reaction mixture of claim 1, wherein the nucleotide sugar is
2 synthesized by an enzymatic pathway that includes one or more enzymes that are expressed
3 from heterologous genes.

1 12. The reaction mixture of claim 11, wherein the recombinant
2 glycosyltransferase is a sialyltransferase, the nucleotide sugar is CMP-sialic acid and the
3 heterologous gene encodes CMP-sialic acid synthetase.

1 13. The reaction mixture of claim 12, wherein the acceptor saccharide is
2 lactose and the product saccharide is sialyllactose.

1 14. The reaction mixture of claim 11, wherein the recombinant
2 glycosyltransferase is a β 1,4-GalNAc transferase and the nucleotide sugar is UDP-GalNAc.

1 15. The reaction mixture of claim 14, wherein the acceptor is lactose and
2 the product saccharide is β 1,4-GalNAc-lactose.

1 16. The reaction mixture of claim 11, wherein the recombinant
2 glycosyltransferase is a galactosyltransferase and the nucleotide sugar is UDP-Gal.

1 17. The reaction mixture of claim 16, wherein the galactosyltransferase is
2 an α 1,3-galactosyltransferase and the product saccharide contains a terminal α 1,3-linked
3 galactose residue.

1 18. The reaction mixture of claim 11, wherein the enzymatic pathway
2 comprises a full or partial sugar nucleotide regeneration cycle.

1 19. The reaction mixture of claim 18, wherein the nucleotide sugar is UDP-
2 GalNAc and the sugar nucleotide regeneration cycle comprises a set of enzymes selected
3 from the group consisting of:

4 UDP-GalNAc epimerase, UDP-GlcNAc pyrophosphorylase, GlcNAc-1-
5 kinase, polyphosphate kinase and pyruvate kinase; and

6 UDP-GalNAc pyrophosphorylase, GlcNAc-1-kinase, polyphosphate
7 kinase and pyruvate kinase.

1 20. The reaction mixture of claim 19, wherein the reaction mixture further
2 comprises a second cell type that produces a nucleotide that is used as a substrate for the
3 sugar nucleotide regeneration cycle.

1 21. The reaction mixture of claim 20, wherein the second cell type
2 comprises an exogenous gene that encodes a nucleotide synthetase polypeptide that catalyzes
3 the synthesis of the nucleotide.

1 22. The reaction mixture of claim 21, wherein the first cell type comprises
2 exogenous genes that encode a) a fusion protein that comprises a polypeptide having 3'-
3 sialyltransferase activity and a polypeptide that has CMP-sialic acid synthetase activity; and
4 b) enzymes that catalyze the synthesis of sialic acid from GlcNAc;

5 and the second cell type comprises an exogenous gene that encodes
6 CMP-synthetase.

1 23. The reaction mixture of claim 21, wherein the first cell type is *E. coli*
2 and the second cell type is yeast or *Corynebacterium*.

1 **24.** The reaction mixture of claim **1**, wherein the first type of cell produces a
2 second recombinant glycosyltransferase that catalyzes the transfer of a sugar from the
3 nucleotide sugar to the product saccharide to form a further glycosylated product saccharide.

1 **25.** The reaction mixture of claim **24**, wherein the nucleotide sugar is UDP-
2 Gal, the first recombinant glycosyltransferase is an β 1,4-galactosyltransferase and the second
3 recombinant glycosyltransferase is an α 1,3-galactosyltransferase.

1 **26.** The reaction mixture of claim **25**, wherein the acceptor saccharide is
2 Glc(R) β -O-R¹, wherein R¹ is -(CH₂)_n-COX; X is selected from the group consisting of OH,
3 OR², -NHNH₂, R is OH or NAc; R² is a hydrogen, a saccharide, an oligosaccharide or an
4 glycon group having at least one carbon atom, and n is an integer from 2 to 18.

1 **27.** The reaction mixture of claim **25**, wherein the UDP-Gal is generated by
2 enzymes that are expressed from exogenous genes that encode UDP-Gal 4' epimerase and
3 UDP-Glc pyrophosphorylase.

1 **28.** The reaction mixture of claim **1**, wherein the cell further comprises: a)
2 an enzymatic system for producing at least a second nucleotide sugar, and b) at least a
3 second recombinant glycosyltransferase that catalyzes transfer of a sugar from the second
4 nucleotide sugar to the product sugar.

1 **29.** The reaction mixture of claim **28**, wherein:
2 the first recombinant glycosyltransferase is a GlcNAc transferase and
3 the first nucleotide sugar is UDP-GlcNAc; and
4 the second recombinant glycosyltransferase is a galactosyltransferase
5 and the second nucleotide sugar is UDP-galactose.

1 **30.** The reaction mixture of claim **29**, wherein the reaction mixture forms
2 lacto-N-neotetraose (LNnT).

1 **31.** The reaction mixture of claim **1**, wherein the reaction mixture also
2 comprises at least a second type of cell that produces a) a second nucleotide sugar, and b) a
3 second recombinant glycosyltransferase that catalyzes the transfer of the sugar from the
4 second nucleotide sugar to the product saccharide.

1 **32.** The reaction mixture of claim **31**, wherein the first glycosyltransferase
2 is a galactosyltransferase and the second glycosyltransferase is a GalNAc transferase.

1 **33.** The reaction mixture of claim **31**, wherein:
2 the first cell type comprises a recombinant β 1,4-GalNAc transferase, a
3 recombinant β 1,4-Gal transferase, UDP-GalNAc and UDP-Gal; and
4 the second cell type comprises a recombinant α 2,3-sialyltransferase and
5 CMP-sialic acid.

1 **34.** The reaction mixture of claim **33**, wherein the CMP-sialic acid is
2 produced from CTP and GlcNAc by an enzymatic system in the second cell type that
3 includes recombinant enzymes CMP-sialic acid synthetase, GlcNAc epimerase, NeuAc
4 aldolase, and CMP-synthetase.

1 **35.** The reaction mixture of claim **33**, wherein the acceptor saccharide is
2 lactosylceramide or lyso-lactosylceramide and the product saccharide is ganglioside GM₂.

1 **36.** The reaction mixture of claim **33**, wherein the second cell type further
2 comprises a recombinant α 2,8-sialyltransferase.

1 **37.** The reaction mixture of claim **36**, wherein the acceptor is
2 lactosylceramide or lyso-lactosylceramide and the product saccharide is GD₂.

1 **38.** The reaction mixture of claim **1**, wherein the reaction mixture also
2 comprises a second type of cell that produces a nucleotide from which is synthesized the
3 nucleotide sugar produced by the first type of cell.

1 **39.** The reaction mixture of claim **38**, wherein nucleotide produced by the
2 second cell type and the corresponding nucleotide sugar are selected from the group
3 consisting of:

4 UTP: UDP-Gal, UDP-GalNAc, UDP-GlcNAc, UDP-Glc, UDP-
5 glucuronic acid, or UDP-galacturonic acid;

6 GTP: GDP-Fuc; and

7 CTP: CMP-sialic acid.

1 **40.** A cell that produces a product saccharide, wherein the cell comprises:
2 a) a recombinant gene that encodes a glycosyltransferase;
3 b) an enzymatic system for forming a nucleotide sugar that is a
4 substrate for the glycosyltransferase; and

5 c) an exogenous saccharide acceptor moiety;
6 wherein the glycosyltransferase catalyzes the transfer of a sugar from
7 the nucleotide sugar to the acceptor moiety to produce the product saccharide.

1 **41.** The cell of claim **40**, wherein the enzymatic system for forming a
2 nucleotide sugar comprises cycle enzymes for regenerating the nucleotide sugar.

1 **42.** The cell of claim **40**, wherein the recombinant gene that encodes a
2 glycosyltransferase is a heterologous gene.

1 **43.** The cell of claim **40**, wherein the cell forms the nucleotide sugar at an
2 elevated level compared to a wild-type cell.

1 44. The cell of claim **43**, wherein the elevated level of nucleotide sugar
2 results from a deficiency in the ability of the cell to incorporate the nucleotide sugar into a
3 polysaccharide normally produced by the cell.

1 45. The cell of claim **44**, wherein the deficiency is due to a reduced level of
2 a polysaccharide glycosyltransferase activity.

1 46. The cell of claim **40**, wherein the product saccharide is produced at a
2 concentration of at least about 1 mM.

1 47. The cell of claim **40**, wherein the enzymatic system for forming a
2 nucleotide sugar comprises an enzyme encoded by a heterologous gene.

1 48. The cell of claim **47**, wherein the enzyme encoded by the heterologous
2 gene is one or more of:

3 a GDP-mannose dehydratase, a GDP-mannose 3,5-epimerase, and a
4 GDP-mannose 4-reductase;

5 a UDP-galactose 4' epimerase;

6 a UDP-GalNAc 4' epimerase;

7 a CMP-sialic acid synthetase;

8 a pyrophosphorylase selected from the group consisting of a UDP-Glc
9 pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a
10 GDP-mannose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase;

11 a kinase selected from the group consisting of myokinase, pyruvate
12 kinase, acetyl kinase, creatine kinase; and
13 pyruvate decarboxylase.

1 49. The cell of claim **48**, wherein the nucleotide sugar is GDP-fucose.

1 50. A cell that produces a sulfated polysaccharide, the cell comprising:

a heterologous gene that encodes a sulfotransferase; and an enzymatic system that produces PAPS

51. The cell of claim 50, wherein the sulfated polysaccharide is selected from the group consisting of heparin sulfate and carragenin.

52. The cell of claim 50, wherein the enzymatic system that produces PAPS comprises one or more enzymes that are expressed from exogenous genes.

1 53. A method of producing a product saccharide, the method comprising
2 contacting a microorganism or plant cell with an acceptor saccharide, wherein the cell
3 comprises:

4 a) an enzymatic system for forming a nucleotide sugar; and
5 b) a recombinant glycosyltransferase which catalyzes the transfer of a
6 sugar from the nucleotide sugar to the acceptor saccharide to produce the product saccharide

54. The method of claim 53, wherein the glycosyltransferase is encoded by a heterologous gene.

1 55. The method of claim 53, wherein the glycosyltransferase is encoded by
2 a gene that is endogenous to the cell and is produced by the cell at an elevated level
3 compared to a wild-type cell.

56. The method of claim 53, wherein the product saccharide is produced at a concentration of at least about 1 mM

1 57. The method of claim 53, wherein the cell is permeabilized.

1 58. The method of claim 53, wherein the cell is an intact cell.

59. The method of claim 53, wherein the enzymatic system for forming a nucleotide sugar comprises an enzyme that is encoded by a heterologous gene.

1 **60.** The method of claim **59**, wherein the enzyme encoded by the
2 heterologous gene is one or more of:
3 a GDP-mannose dehydratase, a GDP-4-keto-6-deoxy-D-mannose 3,5-
4 epimerase, and a GDP-4-keto-6-deoxy-L-glucose 4-reductase;
5 a UDP-galactose 4' epimerase;
6 a UDP-GalNAc 4' epimerase;
7 a CMP-sialic acid synthetase;
8 a pyrophosphorylase selected from the group consisting of a UDP-Glc
9 pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a
10 GDP-mannose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase; a kinase
11 selected from the group consisting of myokinase, pyruvate kinase, acetyl kinase, creatine
12 kinase; and
13 pyruvate decarboxylase.

1 **61.** The method of claim **59**, wherein the enzyme for forming a nucleotide
2 sugar and the glycosyltransferase are expressed as a fusion protein.

1 **62.** The method of claim **61**, wherein the fusion protein comprises a CMP-
2 sialic acid synthetase activity and a sialyltransferase activity.

1 **63.** The method of claim **61**, wherein the fusion protein comprises a
2 galactosyltransferase activity and a UDP-Gal 4' epimerase activity.

1 **64.** The method of claim **61**, wherein the fusion protein comprises a
2 GalNAc transferase activity and a UDP-GlcNAc 4' epimerase activity.

1 **65.** The method of claim **53**, wherein the nucleotide sugar is GDP-fucose
2 and the glycosyltransferase is a fucosyltransferase.

1 **66.** The method of claim **53**, wherein the cell forms the nucleotide sugar at
2 an elevated level compared to a wild-type cell.

1 **67.** The method of claim **66**, wherein the elevated level of nucleotide sugar
2 results from a deficiency in the ability of the cell to incorporate the nucleotide sugar into a
3 polysaccharide normally produced by the cell.

1 **68.** The method of claim **67**, wherein the deficiency is due to a reduced
2 level of a polysaccharide glycosyltransferase activity.

1 **69.** The method of claim **53**, wherein the cell/nucleotide sugar are selected
2 from the group consisting of:

3 *Azotobacter vinelandii*/GDP-Man;
4 *Pseudomonas* sp./UDP-Glc and GDP-Man;
5 *Rhizobium* sp./UDP-Glc, UDP-Gal, GDP-Man;
6 *Erwinia* sp./UDP-Gal, UDP-Glc;
7 *Escherichia* sp./UDP-GlcNAc, UDP-Gal, CMP-NeuAc, GDP-Fuc;
8 *Klebsiella* sp./UDP-Gal, UDP-GlcNAc, UDP-Glc, UDP-GlcNAc;
9 *Hansenula jadinii*/ GDP-Man, GDP-Fuc;
10 *Candida famata*/UDP-Glc, UDP-Gal, UDP-GlcNAc;
11 *Saccharomyces cerevisiae*/UDP-Glc, UDP-Gal, GDP-Man, GDP-
12 GlcNAc; and
13 *X. campestris*/UDP-Glc, GDP-Man.

1 **70.** The method of claim **53**, wherein the cell is *Azotobacter vinelandii*, the
2 nucleotide sugar is GDP-mannose, the acceptor saccharide is lactose, the glycosyltransferase
3 is mannosyl transferase, and the product saccharide is mannosyl lactose.

1 **71.** The method of claim **53**, wherein the cell is *E. coli*, the nucleotide sugar
2 is CMP-sialic acid, the acceptor saccharide is lactose, the glycosyltransferase is a
3 sialyltransferase, and the product saccharide is sialyllactose.

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